Plant Physiol. (1997) 113: 479–485

A 28-Kilodalton Pod Storage Protein of French Bean Plants[1]

Purification, Characterization, and Primary Structure

Pei-Ying Zhong, Tetsutaro Tanaka, Daisuke Yamauchi, and Takao Minamikawa*

Department of Biology, Tokyo Metropolitan University, Minami-ohsawa 1–1, Hachioji, Tokyo 192–03, Japan

When French bean (*Phaseolus vulgaris*) plants were depodded in the early stages of fruit development, relative levels of a specific protein with a relative molecular weight of 28,000 were enhanced in the young pods that formed later. The protein, designated pod storage protein (PSP), was purified from extracts of newly formed pods from plants that had been previously depodded four times at intervals of 2 weeks. Two-dimensional polyacrylamide gel electrophoresis showed the presence of three forms (designated A, B, and C) of PSP with identical electrophoretic mobilities but different charges. The molecular mass of native PSP was estimated by gel filtration to be 67 kD; therefore, the protein was most likely present as a dimer. The antisera raised against forms A and C were cross-reactive with each other. Form B lacked the N-terminal alanine of forms A and C. An expression library from French bean pods was screened using the antiserum against form A, and a full-length cDNA clone was isolated. The cDNA insert included 765 bp potentially encoding a polypeptide with 255 amino acid residues (and a calculated molecular mass of 28,854 D). The amino acid sequence deduced from the PSP cDNA had 65 to 71% identity with soybean (*Glycine max*) vegetative storage protein sequences (P.E. Staswick [1988] Plant Physiol 87: 250–254; and Correction [1989] Plant Physiol 89: 717). Genomic Southern blot analysis suggested that PSP is derived from a single-copy gene.

During fruit development in French bean (*Phaseolus vulgaris*) plants, the accumulation of protein in developing pods precedes that in seeds (Endo et al., 1987). As reported for other legumes such as field pea (Flinn and Pate, 1968), bean (Oliker, 1978), and cowpea (Peoples et al., 1985a, 1985b, 1985c), protein accumulates in French bean seeds during fruit development concurrently with active mobilization of protein and free amino acids in the pods. Thus, a proportion of seed nitrogen is temporarily stored in pods as proteins, amino acids, and probably small peptides, which are then mobilized to support the growth of seeds. We also observed a continued increase in endopeptidase activity throughout the development and subsequent senescence of French bean pods (Endo et al., 1987). The endopeptidase activity is thought to participate in protein mobilization in pods during fruit maturation (Tanaka et al., 1993).

In the course of studies of endopeptidases in pods of French bean plants, we found that when a large number of pods in the early stages of fruit development were removed from a plant, relative levels of a specific protein with an M_r of 28,000 were enhanced in the newly formed pods. The protein accumulated to high levels in the pods of plants that had been previously depodded, whereas a smaller amount was detected in the pods of nontreated plants. It was postulated that enhanced levels of the M_r 28,000 protein resulted from a reduction of plant sink size caused by removing pods in the early stages of fruit development.

In this report, we characterize this protein from French bean pods and designate it PSP. PSP cDNA clones were isolated, and the deduced amino acid sequence showed 65 to 71% identity with the sequences of VSPs from soybean (*Glycine max*) leaves (Staswick, 1988) and 40% identity with the sequences of acid phosphatase-1 from tomato (Erion et al., 1991).

MATERIALS AND METHODS

Dry seeds of French bean (*Phaseolus vulgaris* L. cv Goldstar) and soybean (*Glycine max* cv Mikawajima) were purchased from Sakata Seed (Yokohama, Japan). If not stated otherwise, French bean plants were grown in a phytotron with 13 h of light (190 μE s^{-1} m^{-2}) and 11 h of darkness at 25°C and, at desired developmental stages, maturing fruits were harvested and stored at −20°C until use. For the purification of PSP, pods were collected as follows: just after flowering, all pods were removed from bean plants four times at 2-week intervals, and the few pods that formed thereafter were allowed to grow and were harvested between 22 and 27 d after flowering (stage V; Endo et al., 1987). Leaves were collected from depodded and nontreated plants and stored at −20°C until analysis. Soybean plants were also grown in the phytotron, and when the second trifoliate leaves were fully expanded these were collected and stored at −20°C until use.

[1] This work was supported in part by a grant-in-aid (no. 06304013) from the Ministry of Education, Science, and Culture of Japan.

* Corresponding author; e-mail minamikawa-takao@c.metro-u.ac.jp; fax 81–426–77–2559.

Abbreviations: CBB, Coomassie brilliant blue; 2-ME, 2-mercaptoethanol; PSP, pod storage protein; VSP, vegetative storage protein.

Purification of PSP from French Bean Pods

All manipulations were carried out at 0 to 4°C. Pods (72 g) were powdered with 20 volumes of cold acetone in a homogenizer (model AM-10, Nihonseiki, Tokyo, Japan). The dry powders were homogenized with a chilled mortar and pestle for 40 min in 50 mM potassium phosphate buffer (pH 7.2) containing 10 mM 2-ME. The homogenate was squeezed through two layers of 80-μm nylon cloth and centrifuged at 27,000*g* for 20 min. Solid ammonium sulfate was added to the supernatant to obtain the fraction that was precipitated between 50 and 80% saturation. The precipitated proteins were dissolved in a small volume of 50 mM potassium phosphate buffer (pH 7.2) containing 5 mM 2-ME and dialyzed overnight against the same buffer. A portion (17.6 mg of protein) of the supernatant from the dialyzed solution was applied to a column (1.6 × 15 cm) of DEAE-cellulose (DE-52, Whatman) that had been equilibrated with 50 mM potassium phosphate buffer (pH 7.2) containing 5 mM 2-ME.

The loaded column was first washed with the same buffer and subsequently eluted with a linear gradient of 0 to 0.5 M KCl in the buffer. The eluate was collected in 5-mL fractions at a flow rate of 40 mL h^{-1}, and those fractions containing PSP were combined and lyophilized. A portion (4.4 mg of protein) of the PSP fraction was dissolved in 4 mL of the buffer containing 0.2 M KCl and loaded onto a column (1.6 × 83 cm) of Sephacryl S-200 HR (Pharmacia) that had been equilibrated with the same buffer. The eluate was collected in 5-mL fractions at a flow rate of 12 mL h^{-1}. The fractions containing PSP were combined, dialyzed against water, and lyophilized. The PSP fraction (2.6 mg of protein) was dissolved in a mixed solution of 0.2 mL of the buffer and 0.1 mL of aqueous 80% (v/v) glycerol. The solution was loaded onto a 9% (w/v) acrylamide gel (1 mm thick and 13.5 cm wide) at 4°C.

After electrophoresis, narrow strips of the gel were stained with CBB. Three protein bands, which we called forms A, B, and C, were visualized on the strips. Each band was cut from the gel plate and the protein was electrophoretically recovered from the cut gel using 0.1 M Tris-0.1 M Tricine buffer (pH 8.0) containing 50 mM SDS. The protein solution was dialyzed against water and lyophilized; 0.56, 0.35, and 0.51 mg of proteins were obtained for forms A, B, and C, respectively.

Preparation of Antisera and Protein Immunoblotting

Antiserum against form A or C of PSP was raised in a rabbit by five subcutaneous injections of the purified protein (0.70 or 0.65 mg in total) emulsified with Freund's complete adjuvant at 2-week intervals. The immunospecificity of the antisera against forms A and C was examined by the immunoblot method, as described elsewhere (Tanaka et al., 1993). Protein bands were visualized by enhanced chemiluminescence western blotting detection reagents (Amersham).

Determination of N-Terminal Amino Acid Sequences

Protein sequencing was performed as described by Matsudaira (1987) with some modifications. Each form of PSP was electrophoresed in the SDS gels as described above, and the protein was electroblotted onto a PVDF membrane (Millipore) with 25 mM Tris-boric acid buffer (pH 8.3) containing 20% (v/v) methanol. The membrane was soaked with 10 mM sodium borate containing 25 mM NaCl and stained with CBB. The band at 28 kD was cut out and analyzed by an automatic protein sequencer (model 477A, Applied Biosystems) equipped with an on-line HPLC system for the quantitative identification of phenylthiohydantoin derivatives of amino acids.

RNA Extraction and Northern Blot Analysis

Total RNA was prepared from pods and leaves of depodded and nontreated plants as described previously (Suzuki and Minamikawa, 1985), except the RNA extraction buffer consisted of 200 mM Tris-HCl (pH 8.5), 300 mM NaCl, 20 mM EDTA, and 1% SDS and phenol saturated with 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA.

Northern blot analysis was performed as described previously (Ishibashi and Minamikawa, 1990), except that a nylon membrane filter (Hybond-N, Amersham) was used instead of a nitrocellulose sheet. A ^{32}P-labeled DNA probe was prepared from the λPSP1 insert using a random primer labeling kit (Takara Shuzo, Kyoto, Japan). The filter was washed with 0.5× SSC containing 0.1% SDS for 90 min at 42°C and exposed to x-ray film.

cDNA Cloning and Sequencing

Pods at stage IV were collected from the depodded French bean plants grown at the university experimental farm from May to July, and total RNA was prepared from the pods as described above. Poly(A) RNA was isolated from the total RNA by oligo(dT)-cellulose column chromatography (Aviv and Leder, 1972). Double-stranded cDNA was synthesized by the cDNA Synthesis System Plus (Amersham) according to the manufacturer's instructions. The synthesized cDNA was inserted into the λgt11 vector (Huynh et al., 1985). cDNA clones for PSP were selected from the library using the antiserum against form A of PSP as described by Huynh et al. (1985), with the exception that the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) was used instead of ^{125}I-labeled protein A. The clones obtained by the screening were examined by epitope selection (Weinberger et al., 1985). Plaque hybridization was carried out as described by Sambrook et al. (1989). The nucleotide sequence of the cDNA for PSP was determined in both directions by the dideoxynucleotide chain-termination method using the pUC118 system (Sambrook et al., 1989).

Southern Blot Analysis

nDNA from French bean plants was prepared as described previously (Yamauchi et al., 1989). Southern blot-

ting was carried out according to the method of Sambrook et al. (1989).

Other Methods

A crude extract of leaves from the depodded plants was prepared by the same method as for the pods. Protein contents were measured by the method of Bradford (1976) using BSA as a standard.

A partially purified VSP preparation (1.1 mg of protein) was obtained by gel filtration of an extract from leaves (30 g) of soybean plants according to the method of Wittenbach (1983). Glycochains in polypeptides were detected by the periodic acid-Schiff staining method (Kondo et al., 1991). After SDS-PAGE on a 1-mm-thick 12.5% gel, proteins in the gel were transferred to a PVDF transfer membrane (Millipore) and stained with reagents (G.P. Sensor, Seikagaku Kogyo, Tokyo, Japan) according to the manufacturer's instructions.

RESULTS

Accumulation of PSP in Pods of French Bean Plants That Had Been Depodded

When French bean plants were depodded early in fruit development, relative levels of a specific M_r 28,000 protein were enhanced in the few pods that formed later. Smaller amounts of the protein were detected in pods of nontreated control plants (Fig. 1). The protein in newly formed pods of depodded plants grown in a phytotron accumulated to higher levels than when the plants were grown at an experimental farm. To compare levels of the M_r 28,000 protein in pods of depodded and nontreated plants, relative

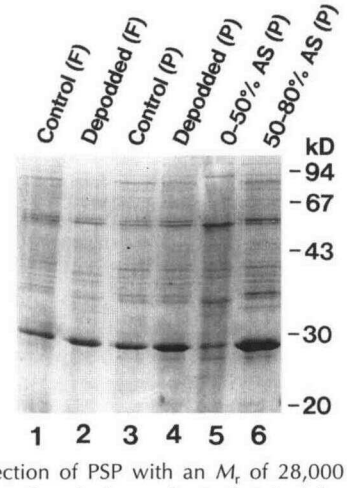

Figure 1. Detection of PSP with an M_r of 28,000 in French bean plants. Extracts of pods from plants that had been depodded as described in "Materials and Methods" were analyzed by SDS-PAGE (lanes 2 and 4). Pods from nontreated control plants were analyzed in the same manner (lanes 1 and 3). Ammonium sulfate (AS) fractions (0–50 and 50–80% saturation) were prepared from a crude extract of pods from the plants that had been depodded and analyzed by SDS-PAGE (lanes 5 and 6). Plants were grown in a phytotron (P) or at the university experimental farm (F). The sample solution was treated with an equal volume of SDS sample buffer (Laemmli, 1970), and 15 μg of protein was loaded on each lane.

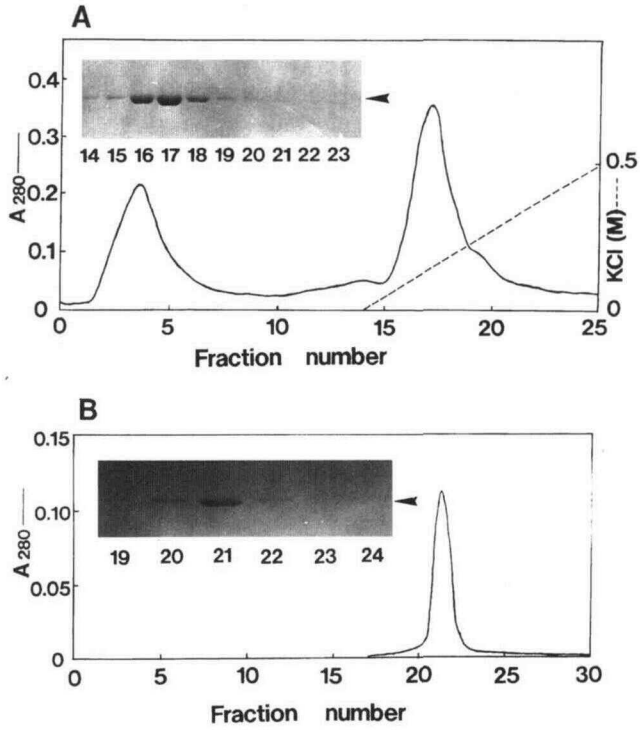

Figure 2. Purification of PSP from pods of depodded plants. A, Separation of PSP by a column of DEAE-cellulose chromatography. A 50 to 80% saturated ammonium sulfate fraction from crude extracts of the pods was applied to the column (DE-52, Whatman), which had been equilibrated with 50 mM potassium phosphate buffer (pH 7.2) containing 10 mM 2-ME. The column was eluted with a linear gradient of 0 to 0.5 M KCl in the same buffer, and 5-mL fractions were collected at a flow rate of 40 mL h^{-1}. Inset, SDS-PAGE pattern of tubes 14 to 23 (25 μL from each tube). Arrowhead, M_r 28,000 protein. B, Separation of PSP by gel-filtration column chromatography on Sephacryl S-200. A combined fraction (4.8 mg of protein) of tubes 14 to 23 obtained from the DEAE-cellulose column was applied onto the column and eluted with the buffer containing 0.2 M KCl at a flow rate of 12 mL h^{-1}. The eluate was collected in 5-mL fractions. Inset, SDS-PAGE pattern of tubes 19 to 24 (25 μL from each tube). The gels were stained with CBB. Arrowhead, M_r 28,000 protein.

amounts of protein were assessed by scanning the CBB-stained bands with a densitometer after SDS-PAGE. The results of six independent experiments indicated that the amount of M_r 28,000 protein accumulated per gram fresh weight of pod from depodded plants grown in the phytotron was 159 ± 11% (SE) of that from corresponding nontreated plants. The value from four experiments was 132 ± 8% in the case of plants grown at the experimental farm. Enhanced levels of the M_r 28,000 protein were also observed in pods that were formed at an early stage of fruit development when pods formed later were removed. We designated this M_r 28,000 protein as PSP.

Purification and Characterization of PSP

PSP was purified from extracts of pods from plants that had been depodded in the early stages of fruit development. The crude extracts were fractionated by ammonium

sulfate, and PSP was found to be enriched in the fraction of 50 to 80% saturation (Fig. 1, lanes 5 and 6). Elution patterns of PSP from columns of DEAE-cellulose and Sephacryl S-200 are presented in Figure 2. The final gel-filtration step provided an apparently homogeneous PSP preparation, as determined by CBB staining after SDS-PAGE (Fig. 2B). However, analysis of the preparation by two-dimensional PAGE revealed three forms (A, B, and C) of PSP with identical mobilities (M_r 28,000) but different net charges (Fig. 3A). Nondenaturing PAGE (Hedrick and Smith, 1986) revealed that the molecular mass of forms A and C was approximately 67 kD (data not shown), indicating that PSP was probably present as a dimer. Forms A and C shared at least 16 identical amino acid residues among the N-terminal 20 residues (Fig. 3B). The nine N-terminal amino acids of form B were identical with those of forms A and C, but Ala-1 of forms A and C was lacking in form B.

Nucleotide Sequence of PSP cDNA

A cDNA expression library from French bean pods of stage V (Tanaka et al., 1993) was immunologically screened, and nine clones were selected. However, only one clone, termed λP5, reacted specifically with the antiserum against PSP in epitope selection (Weinberger et al., 1985). This clone carried a cDNA insert of approximately

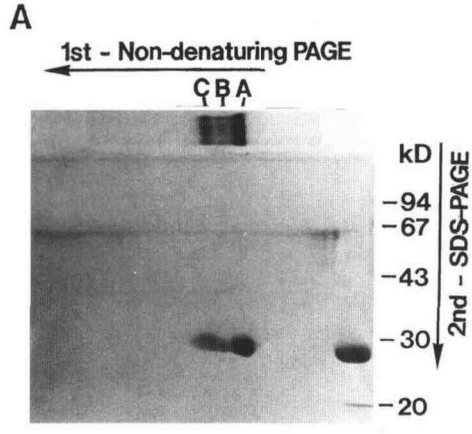

Figure 3. Characterization of three forms of PSP. A, Two-dimensional PAGE pattern of PSP. The PSP fraction from the gel-filtration column (4.5 μg of protein) was analyzed by two-dimensional PAGE. The first dimension was 9% nondenaturing PAGE and the second was 12.5% SDS-PAGE. Arrows indicate the direction of migration. The first- and second-dimensional PAGE patterns were attached to the top and the right of the two-dimensional PAGE gel, respectively. The gel was stained with CBB. Molecular masses are shown on the right. B, N-terminal amino acid sequences of the three forms of PSP. The amino acid sequences were determined as described in "Materials and Methods." X, Undetermined amino acids.

```
                                                G ATAACAAGAA GAAGAAGCAG TGAGTGAGTT   -1

ATGAAGTGCC TCGTGTTCTT TGTTGCTGCA GTTTTGGTGG CATCTCAATG CCATGGCGCT    60
M  K  C  L   V  F  F   V  A  A   V  L  V  A  ▲S  Q  C   H  G  A     20

TCCTTCCGAA GCTTCCCTCT GAGCATGACA ACTGGTTATG GCGACGGTGC TAGTGACACG   120
S  F  R  S   F  P  L   S  M  T   T  G  Y  G   D  G  A   S  D  T     40

GAGGTACGAT GCGCCAGTTG GAGGCTTGCG GTGGAAGCAC AGAACATCTT CGGCTTTGAA   180
E  V  R  C   A  S  W   R  L  A   V  E  A  Q   N  I  F   G  F  E     60

ACCATTCCTC AACAGTGCGT AGACGCTACA GCCAACTACA TCGAAGGAGG ACAATACAGA   240
T  I  P  Q   Q  C  V   D  A  T   A  N  Y  I   E  G  G   Q  Y  R     80

TCAGACTCCA AAACAGTTAA CCAACAGATT TACTTTTTCG CTAGAGATCG CCATGTCCAT   300
S  D  S  K   T  V  N   Q  Q  I   Y  F  F  A   R  D  R   H  V  H    100

GAGAACGATG TCATTCTGTT CAACATAGAT GGAACTGCAC TCTCCAATAT CCCATACTAT   360
E  N  D  V   I  L  F   N  I  D   G  T  A  L   S  N  I   P  Y  Y    120

TCTCAACATG GATATGGATC GGAGAAATTC GATTCCGAAC GCTATGACGA AGAGTTTGTT   420
S  Q  H  G   Y  G  S   E  K  F   D  S  E  R   Y  D  E   E  F  V    140

AACAAGGGTG AGGCACCAGC ATTGCCTGAG ACTCTCAAGA ATTACAACAA ACTGGTGTCT   480
N  K  G  E   A  P  A   L  P  E   T  L  K  N   Y  N  K   L  V  S    160

CTTGGCTACA AGATTATCTT CTTAAGTGGA AGGCTAAAGG ACAAAAGAGC TGTAACTGAA   540
L  G  Y  K   I  I  F   L  S  G   R  L  K  D   K  R  A   V  T  E    180

GCCAACCTAA AGAAGGCTGG TTACAACACA TGGGAGAAGT TGATTCTCAA GGACCCATCT   600
A  N  L  K   K  A  G   Y  N  T   W  E  K  L   I  L  K   D  P  S    200

AACAGTGCTG AAAATGTAGT TGAATACAAA ACAGCTGAGA GAGCGAAGCT GGTGCAGGAG   660
N  S  A  E   N  V  V   E  Y  K   T  A  E  R   A  K  L   V  Q  E    220

GGTTACAGAA TCGTTGGAAA TATTGGAGAC CAGTGGAACG ATCTGAAGGG AGAGAACAGA   720
G  Y  R  I   V  G  N   I  G  D   Q  W  N  D   L  K  G   E  N  R    240

GCAATAAGGA GCTTTAAGCT GCCTAATCCC ATGTACTACA CTAAGTAGTT CATCTCATGG   780
A  I  R  S   F  K  L   P  N  P   M  Y  Y  T   K                    255

CTCTAGCTTG CTCCTATATA TGTATCAAAT AAGTAATCCA AAGAGGAGCA TTGCCTGTGT   840

ATCAACTAGT CACCTAAGAG ACTCCACTAC TTTGTTTTCT CTTTGTGGCT TTAATAAATT   900

ATGAAACTTT CTCAAAAAAA                                               920
```

Figure 4. Complete nucleotide sequence of the λPSP1-cDNA insert and the deduced amino acid sequence. Amino acid residues identical with the N-terminal amino acid sequences of forms A and C of PSP are double-underlined. Nucleotides are numbered in a 5′ to 3′ direction beginning with the first nucleotide of the initiation codon. Amino acids are numbered from the initiation Met. The arrowhead indicates a putative signal peptide cleavage site. A putative polyadenylation signal sequence is underlined. The DNA Data Bank of Japan accession number for the nucleotide sequence of λPSP1 cDNA is D50094.

0.6 kb and the sequence from the insert lacked a poly(dA) tract, suggesting that it represented a partial-length cDNA for PSP. Another cDNA library from pods of stage III was then screened with λP5 cDNA as a probe. Several clones were obtained and we chose a clone carrying the longest cDNA insert. This clone, designated λPSP1, included 765 bp of an open reading frame potentially encoding a polypeptide with 255 amino acid residues (calculated molecular mass 28,854 D), 31 bp of a 5′ untranslated region, and a 148-bp 3′ untranslated region (Fig. 4). The N-terminal amino acid sequence of forms A and C of PSP corresponded to amino acid residues 37 to 52. The deduced amino acid sequence of the λPSP1 cDNA has 71 and 65% identity with those of VSP-α and VSP-β of soybean, respectively (Rhee and Staswick, 1992a, 1992b), and 40% identity with acid phosphatase-1 of tomato (Erion et al., 1991). No putative glycosylation sites were contained in the deduced amino acid sequence of λPSP1 cDNA (Fig. 4), although VSPs (Wittenbach, 1983) and acid phosphatase-1 (Paul and Williamson, 1987) are glycoproteins. In fact, PSP was not stained with the periodic acid-Schiff reagent, whereas VSP-α (27 kD) and VSP-β (29 kD) were stained (Fig. 5).

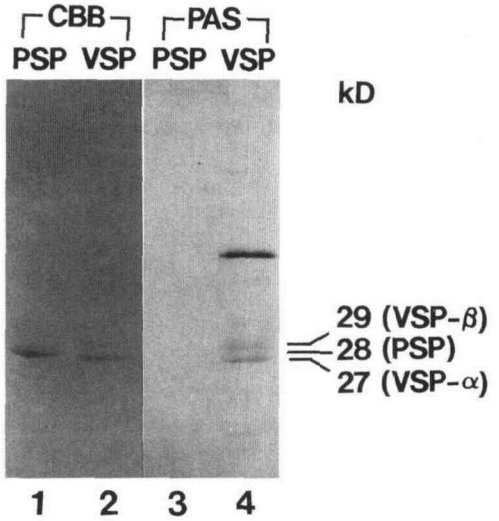

Figure 5. Detection of glycochains in PSP from French bean pods and VSPs from soybean leaves. Lanes 1 and 3, 3 and 2.5 μg of purified PSP, respectively; lanes 2 and 4, 2.4 and 5 μg of protein of partially purified VSP, respectively. After separation on 12.5% SDS-PAGE, gels were stained with CBB and the periodic acid-Schiff (PAS) reagent.

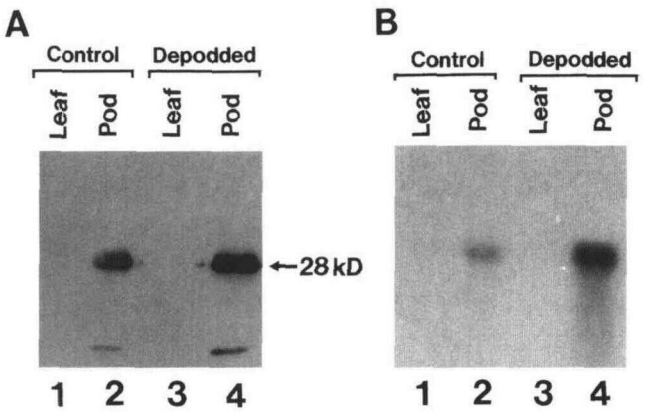

Figure 6. Expression of PSP and PSP mRNA in pods and leaves of depodded and nontreated French bean plants. A, Immunoblot analysis of PSP. Extracts of the expanded leaves and pods at stage V were prepared from nontreated control plants (lanes 1 and 2) and depodded plants (lanes 3 and 4). The extracts (5 μg protein/lane) were analyzed by SDS-PAGE followed by immunoblotting using the antiserum against form A of PSP. The arrow points to the 28-kD PSP. B, Northern blot analysis. Total RNA was prepared from leaves and pods of control plants (lanes 1 and 2) and depodded plants (lanes 3 and 4) at stage V. RNA (10 μg) was glyoxalated and separated by electrophoresis on a 1.4% agarose gel, blotted onto a nylon filter, and hybridized with the ^{32}P-labeled cDNA insert of λPSP1.

Levels of PSP and PSP mRNA in Pods and Leaves

Immunocompetition experiments demonstrated that the antisera raised against forms A and C were cross-reactive (data not shown). In the present studies, antiserum against form B could not be raised, presumably because of the small quantity of form B prepared. The immunoblot analysis using the antiserum against form A of PSP indicated that the depodding treatment enhanced the accumulation of PSP in newly formed pods (Fig. 6A). Immunoblot analysis also demonstrated no accumulation of PSP in leaves from either depodded or nontreated French bean plants, whereas levels of PSP were enhanced in pods of depodded plants at stage V compared with nontreated plants (Fig. 6A). Northern blot analysis with the λPSP1 cDNA probe for the same plant samples indicated that PSP mRNA was detected in pods but not in leaves (Fig. 6B). The mRNA level was enhanced in pods of depodded plants compared with nontreated control plants.

Genomic Southern Blot Analysis

Genomic Southern blot analysis was performed to estimate the copy number of the PSP gene using the λPSP1 cDNA as a probe (Fig. 7). The cDNA hybridized with one (*Pst*I, 6.4 kb; *Xba*I, 9.5 kb; and *Kpn*I, 14.8 kb) or two fragments (*Hin*dIII, 1.6 and 0.8 kb). The results are consistent with there being a single copy of the PSP gene in French bean plants, although it is necessary to propose a *Hin*dIII site in an intron of the gene, since the cDNA lacks any *Hin*dIII site.

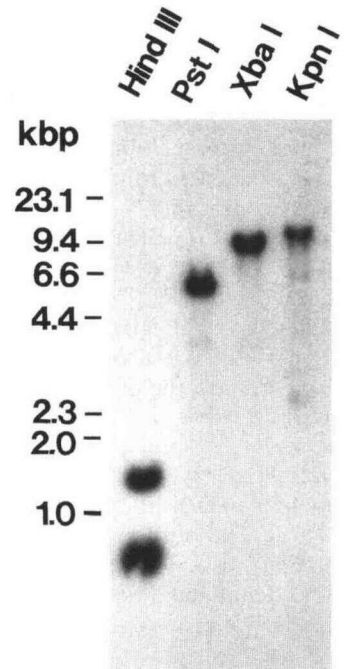

Figure 7. Southern blot analysis of nDNA from French bean plants. The nDNA (10 μg) from French bean plants digested with *Hin*dIII, Pst I, *Xba*I, or *Kpn*I was separated on a 0.8% agarose gel and blotted onto a nylon filter, which was then hybridized with the ^{32}P-labeled cDNA insert of λPSP1.

DISCUSSION

Enhanced levels of PSP in pods of French bean plants were first observed during studies of endopeptidases in

senescing pods (Tanaka et al., 1993). When extracts from newly formed pods of plants that had been largely depodded were analyzed by SDS-PAGE, we found that a M_r 28,000 band on gels stained with CBB was more intense than in extracts of nontreated plants. Removal of pods early in fruit development may lower overall sink strength, and, as a consequence, this specific protein may accumulate in newly formed pods. In the present studies, we isolated and characterized the M_r 28,000 protein and sequenced its cDNA.

The cDNA of PSP contains a translated region coding for a polypeptide with 255 amino acid residues (Fig. 4). From the hydrophobicity of the amino acid sequence, it is predicted that a signal peptide is cleaved between Ala-14 and Ser-15. However, the N-terminal amino acid of forms A and C of PSP corresponded to Ala-37, suggesting that further posttranslational processing of the PSP precursor takes place in the ER or after the precursor is transported from it. Two-dimensional PAGE analysis (Fig. 3A) demonstrated the presence of three forms of PSP, each with an identical M_r (28,000) and a different net charge. Thus, the native PSP of 67 kD was probably present as a dimer. The three forms of PSP may be encoded by a single copy of the gene (Fig. 7). Form B lacked the N-terminal Ala of forms A and C (Fig. 3B). It remains undetermined, however, whether this is due to posttranslational processing at a site different from that of forms A and C, or whether it results from action of an aminopeptidase on form A or C. PSP is apparently not glycosylated (Fig. 5), and hence the different charges between the three forms are probably not attributable to glycochains of the polypeptides.

Removal of pods from soybean plants induces accumulation of a specific leaf protein termed VSP (Staswick, 1988, 1990). VSP consists of two similar polypeptides, VSP-α and VSP-β, which are derived from different genes (Rhee and Staswick, 1992a, 1992b), in contrast to the apparent single copy of the PSP gene in French bean plants (Fig. 7). The deduced amino acid sequence of the PSP cDNA was found to have 71 and 65% identity with those of VSP-α and VSP-β of soybean, respectively (Rhee and Staswick, 1992a, 1992b). PSP also had 40% sequence identity with tomato acid phosphatase-1 (Erion et al., 1991). The amino acid sequences of acid phosphatase-1 and VSP share about 45% identity.

Although PSP and VSP are similar in amino acid sequence and molecular mass, the two proteins are different in terms of induced synthesis and accumulation in plants. PSP was synthesized in remaining or newly formed pods but was not detectable in leaves of the depodded plants, as shown by immunoblot analysis (Fig. 6A). VSP accumulates to high levels in leaves of depodded soybean plants (Staswick, 1990). PSP mRNA was not detected by northern blotting in leaves of depodded or nontreated plants, but its level was increased in pods of depodded plants. The level of PSP mRNA was enhanced more notably than the level of the PSP protein in pods of depodded plants at stage V (Fig. 6). This difference between PSP and its mRNA might be due to differences in their stage-dependent accumulation and also in their stability in the pods. Thus, PSP is probably synthesized to a considerable extent even in pods of nontreated French bean plants, and its content increases by 32 to 59% following depodding, whereas synthesis of VSP is more conspicuously induced in leaves of depodded soybean plants.

The accumulation of PSP in the pods is most abundant in a late stage (stage V; data not shown) of fruit development, but most active accumulation of VSP occurs in an intermediate stage (Staswick, 1989). The synthesis of VSP is clearly induced by jasmonic acid and its methyl ester (Anderson et al., 1989; Staswick, 1990), but our preliminary investigations showed that exogenously applied methyl jasmonate was not effective in enhancing the accumulation of PSP (P.-Y. Zhong, D. Yamauchi, and T. Minamikawa, unpublished data). We thus propose that PSP is a novel type of protein and its accumulation in pods is enhanced by the reduction in competing sinks associated with fruit removal.

Received July 29, 1996; accepted November 4, 1996.

LITERATURE CITED

Anderson JM, Spilatro SR, Klauer SF, Franceschi VR (1989) Jasmonic acid-dependent increase in the level of vegetative storage proteins in soybean. Plant Sci **62:** 45–52

Aviv H, Leder P (1972) Purification of biologically active globulin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA **69:** 1408–1412

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem **72:** 248–254

Endo M, Minamikawa T, Yamauchi D, Mitsuhashi W (1987) Nitrogen mobilization and peptidase activities in pods of maturing *Phaseolus vulgaris* fruits. J Exp Bot **38:** 1988–1995

Erion JL, Ballo B, May L, Bussell J, Fox T, Thomas SR (1991) Isolation and characterization of a tomato acid phosphatase complementary DNA associated with nematode resistance. Plant Physiol **97:** 1462–1469

Flinn AM, Pate JS (1968) Biochemical and physiological changes during maturation of fruit of the field pea (*Pisum arvense* L.). Ann Bot **32:** 479–495

Hedrick JL, Smith AJ (1986) Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. Arch Biochem Biophys **126:** 155–164

Huynh TV, Young RA, Davis RW (1985) Constructing and screening cDNA libraries in λgt10 and λgt11. *In* DM Glover, ed, DNA Cloning, Vol 1. IRL Press, Oxford, UK, pp 49–78

Ishibashi N, Minamikawa T (1990) Molecular cloning and characterization of stored mRNA in cotyledons of *Vigna unguiculata*. Plant Cell Physiol **31:** 39–44

Kondo M, Harada H, Sunada S, Yamaguchi T (1991) Increased selectivity in the detection of glycoproteins on nitrocellulose membranes by washing with sodium hydroxide solution. Electrophoresis **12:** 685–686

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

Matsudaira P (1987) Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J Biol Chem **262:** 10035–10038

Oliker M, Poljakoff-Mayber A, Mayer AM (1978) Changes in weight, nitrogen accumulation, respiration and photosynthesis during growth and development of seeds and pods of *Phaseolus vulgaris*. Am J Bot **65:** 366–371

Paul EM, Williamson VM (1987) Purification and properties of acid phosphatase-1 from a nematode resistant tomato cultivar. Plant Physiol **84:** 399–403

Peoples MB, Atkins CA, Pate JS, Murray DR (1985a) Nitrogen nutrition and metabolic interconversions of nitrogenous solutes in developing cowpea fruits. Plant Physiol **77:** 382–388

Peoples MB, Pate JS, Atkins CA (1985b) The effect of nitrogen source on transport and metabolism of nitrogen in fruiting plants of cowpea (*Vigna unguiculata* [L.] Walp.). J Exp Bot **36:** 567–582

Peoples MB, Pate JS, Atkins CA, Murray DR (1985c) Economy of water, carbon, and nitrogen in the developing cowpea fruit. Plant Physiol **77:** 142–147

Rhee Y, Staswick PE (1992a) Nucleotide sequence of a soybean vegetative storage protein *vspA* gene. Plant Physiol **98:** 792–793

Rhee Y, Staswick PE (1992b) Nucleotide sequence of a soybean vegetative storage protein *vspB* gene. Plant Physiol **98:** 794–795

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Staswick PE (1988) Soybean vegetative storage protein structure and gene expression. Plant Physiol **87:** 250–254; and Correction, Plant Physiol **89:** 717

Staswick PE (1989) Preferential loss of an abundant storage protein from soybean pods during seed development. Plant Physiol **90:** 1252–1255

Staswick PE (1990) Novel regulation of vegetative storage protein genes. Plant Cell **2:** 1–6

Suzuki Y, Minamikawa T (1985) On the role of stored mRNA in protein synthesis in embryonic axes of germinating *Vigna unguiculata* seeds. Plant Physiol **79:** 327–331

Tanaka T, Minamikawa T, Yamauchi D, Ogushi Y (1993) Expression of endopeptidase (EP-C1) in *Phaseolus vulgaris* plants. Plant Physiol **101:** 421–428

Weinberger C, Hollenberg SM, Ong ES, Harmon JM, Brower ST, Cidlowski J, Thompson EB, Rosenfeld MG, Evans RM (1985) Identification of human glucocorticoid receptor complementary DNA clones by epitope selection. Science **228:** 740–742

Wittenbach VA (1983) Purification and characterization of a soybean leaf storage glycoprotein. Plant Physiol **73:** 125–129

Yamauchi D, Nakamura K, Asahi T, Minamikawa T (1989) Nucleotide sequence of cDNA for concanavalin A from *Canavalia gladiata* seeds. Plant Cell Physiol **30:** 147–150